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A new genus of proteocephalid tapeworm (Cestoda) from the marbled swamp eel *Synbranchus marmoratus* Bloch (Synbranchiformes: Synbranchidae) in the River Paraná basin, Argentina

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Abstract: *Synbranchiella* gen. n. is proposed to accommodate *Synbranchiella mabelae* sp. n. (Proteocephalidae: Monticelliinae) from the intestine of the marbled swamp eel *Synbranchus marmoratus* Bloch, in the River Colastiné, a tributary of the middle River Paraná in Argentina. The new genus is placed in the Monticelliinae because of the cortical position of the genital organs. It differs from all known monticelliine genera by the following combination of characters: (i) scolex robust, with a conical apex, without metascolex; (ii) biloculate suckers with a conspicuous septum separating unequally-sized loculi and a robust non-adherent area, lacking free posterior margin; (iii) vitelline follicles in two narrow lateral bands, extended throughout the nearly entire proglottid length; (iv) vagina always anterior to the cirrus-sac, with an inconspicuous vaginal sphincter; (v) a genital pore pre-equatorial. Scanning electron microscopy revealed three types of microtriches on the tegument surface: acicular and capiliform filitriches and gladiate spinitriches. A phylogenetic analysis of the large subunit nuclear ribosomal RNA gene (*lsr*DNA, D1–D3 domains) confirms that *S. mabelae* represents an independent lineage within a large clade comprised mainly from Neotropical taxa parasitising catfishes. This is the second proteocephalidean cestode described from a Neotropical synbranchiform fish host.

Keywords: Proteocephalidae, Monticelliinae, taxonomy, morphology, phylogenetic analysis, freshwater, Neotropical Region

In the Neotropical Region, the number of species of cestodes of the order Proteocephalidea Mola, 1928 (currently part of the Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014) is about one hundred but only 16 species of them occur in non-siluriform fishes of the orders Atheriniformes (1 sp.), Characiformes (7 spp.), Gymnotiformes (3 spp.), Perciformes (4 spp.) and Synbranchiformes (1 sp.) (Alves et al. 2017a).

During a survey of the helminth fauna of fishes from the River Paraná basin, specimens of a hitherto undescribed proteocephalidean species were collected from the intestine of the marbled swamp eel (*Synbranchus marmoratus* Bloch) (Synbranchiformes: Synbranchidae) and subjected to morphological and molecular analyses (*lsr*DNA, D1–D3 domains). These tapeworms were assigned to the Monticelliinae Mola, 1929, but could not be allocated to any of the known monticelliine genera. Therefore, a new genus is proposed to accommodate the new species described herein.

MATERIALS AND METHODS

Seventy-three specimens of *Synbranchus marmoratus* were caught by local fishermen in December 2009 and 2011 from the River Colastiné, Santa Fe Province, and in January, February and April 2010 and December 2011 from the River Paraná-Guazú, Entre Ríos Province, Argentina. Worms found in the intestine were removed, cleaned in saline, fixed in hot 4% formaldehyde solution and subsequently stored in 70% ethanol. Before this fixation for morphological observations, posteriormost proglottids of two specimens were excised and placed in molecular-grade 96–99% ethanol for sequencing; hologenophore was preserved as a voucher (see Pleijel et al. 2008 for terminology).

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Entire tapeworms were stained with Langeron's alcoholic hydrochloric carmine (Langeron 1949), differentiated in acid ethanol, dehydrated through a graded ethanol series, cleared in beechwood creosote and mounted in Canada balsam. Details of the internal anatomy were determined from thick, hand-cut cross serial sections of proglottids stained with Langeron's alcoholic hydrochloric carmine. Spontaneously laid eggs were fixed in hot 4% formaldehyde solution and measured and illustrated in distilled water.

Pieces of two specimens of the new species were prepared for scanning electron microscopy (SEM) as follows: worms were postfixed in 1% osmium tetroxide, dried with hexamethyldisilazane (Riedel-De Haën®, Hannover, Germany), mounted on stubs with adhesive tape, sputter coated with gold in a Thermo VG Scientific Polaron SC 7630 and examined with a Philips XL 30 scanning electron microscope. The types and distribution of microtriches were studied on the scolex, proliferation zone (neck) and immature proglottids. Measurements of the microtriches were taken from photomicrographs. Microthrix terminology follows Chervy (2009). Unless otherwise stated, all measurements are given in micrometres, with the range followed by mean and total number of measurements (n) in parentheses. For two-dimensional measurements, length is given before width. The relative size of the ovary was calculated according to de Chambrier et al. (2012). Illustrations were made with the aid of a camera lucida attached to a Zeiss Axioscope microscope equipped with differential interference contrast optics.

Total genomic DNA was extracted using a QIAamp DNA Blood kit (QIAGEN, Hilden, Germany) following manufacturer's instructions. The protocol for PCR amplification of the large subunit nuclear ribosomal RNA gene (*lsr*DNA, D1–D3 domains) and sequencing were done as described in Brabec et al. (2012). Contiguous sequences were assembled using Geneious version R8 (http://www.geneious.com/; Kearse et al. 2012) and submitted to GenBank. The newly generated sequence of *lsr*DNA was aligned with related sequences retrieved from the GenBank database (see Table 1), using the E-INS-i algorithm of the program MAFFT (Katoh and Standley 2013) implemented in Geneious. The number of parsimony-informative characters was determined using PAUP* version 4a147 (Swofford 2002). The alignment was trimmed to match the shortest sequence and ambiguously aligned positions were manually excluded from subsequent analyses.

Phylogenetic reconstructions were performed with the Maximum likelihood (ML) and the Bayesian inference (BI) criteria, based on GTR + I + Γ model, predicted as best estimator by the small sample size corrected Akaike Information Criterion implemented in PartitionFinder v. 1.1.1 (Lanfear et al. 2012). The best ML estimate was obtained from 100 searches in the program GARLI ver. 2.01 (Zwickl 2006) using default settings and the nodal support was evaluated by running tree searches on each of the 100 bootstrap replicates in GARLI. A BI tree was constructed using MrBayes ver. 3.2 (Ronquist et al. 2012) running two independent MC3 runs of 4 chains (one cold, three heated) for 5 million generations (ngen = 5,000,000), sampling tree topologies every 1,000th generation (samplefreq = 1,000) and the first 500 samples were discarded as burn-in (burninfrac = 0.10). Tracer v.1.6 (Rambaut et al. 2014) was used to check the convergence and mixing of different parameters and to confirm that the effective sample size (ESS) of each parameter was adequate to provide reasonable estimates of the variance in model parameters (i.e. ESS values > 200).

Holotype was deposited in the Helminthological collection of the Institute of Parasitology of the Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS) and paratypes at the Parasitological Collection of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina (MACN-Pa). For comparative purpose, the following monticelliine tapeworms that possess biloculate suckers were studied: type and voucher specimens of *Chambriella megacephala* (Woodland, 1934), *Riggenbachiella amazonense* Alves, de Chambrier, Luque et Scholz, 2017 and *R. paranaense* (Pavanelli et Rego, 1989) by one of the authors (PVA) (see Alves et al. 2017b for the complete list of host and localities).

RESULTS

Synbranchiella gen. n.

ZooBank number for genus:
urn:lsid:zoobank.org:act:953C7E30-DCE7-4EC7-8313-6151134D0BDC

Diagnosis. Proteocephalidea, Proteocephalidae, Monticelliinae. Testes, ovary, vitelline follicles and uterus cortical. Medium-sized worms, flattened dorsoventrally. Strobila with acraspedote proglottids. Scolex subspherical to quadrangular, apex conical to slightly globose, without apical organ. Metascolex absent. Suckers biloculate, robust, with conspicuous septum separating loculi, lacking free posterior margin. Non-adherent area of suckers conspicuously developed. Internal longitudinal musculature formed by a few, small, sparsely distributed bundles of muscle fibres. Proliferation zone (neck) narrower than scolex. Testes cortical, arranged in one irregular field and one layer. Cirrus-sac thin-walled, elongated to pyriform. Genital pore pre-equatorial, irregularly alternating. Genital atrium present. Ovary cortical, butterfly-shaped, slightly lobulated. Vagina anterior to cirrus-sac, surrounded by small terminal vaginal sphincter near genital atrium. Vitelline follicles cortical, arranged in two narrow lateral bands. Uterine stem and uterine branches cortical. Uterine development of type 2 (*sensu* de Chambrier et al. 2004a). Parasites of Neotropical synbranchiform fish (Synbranchidae).

Type and only species: *Synbranchiella mabelae* sp. n.
Etymology: The new genus is named after the generic name of the host and should be treated as feminine.

Differential diagnosis. The new genus is placed in the Monticelliinae based on the position of the internal organs in relation to the inner longitudinal musculature (Schmidt 1986, Rego 1994, de Chambrier et al. 2009). The subfamily currently includes ten genera parasitising freshwater fishes in the Neotropics, *Ageneiella* de Chambrier et Vaucher, 1999; *Chambriella* Rego, Chubb et Pavanelli, 1999; *Choanoscolex* La Rue, 1911; *Goezeella* Fuhrmann, 1916; *Manaosia* Woodland, 1935; *Monticellia* La Rue, 1911; *Regoella* Arredondo, de Chambrier et Gil de Pertierra, 2013; *Riggenbachiella* Alves, de Chambrier, Luque et Scholz, 2017; *Spasskyellina* Freze, 1965 and *Spatulif-*

Table 1. List of cestode specimens whose sequences of the large subunit nuclear ribosomal RNA gene (*lsr*DNA, D1–D3 domains) were included in the analyses. Genbank accession number in bold indicates the sequence generated as part of this study.

Taxon	Host species	Voucher Acc. No.†	GenBank Acc. No.	Reference
Ageneiella brevifilis de Chambrier et Vaucher, 1999	*Ageneiosus inermis* (Linnaeus)	21841	AJ388600	Zehnder and Mariaux 1999
Amphoteromorphus ninoi Carfora, de Chambrier et Vaucher, 2003	*Brachyplatystoma filamentosum* (Lichtenstein)	22239	AJ388624	de Chambrier et al. 2004a
Amphoteromorphus peniculus Diesing, 1850	*Brachyplatystoma rousseauxii* (Castelnau)	60052	KP729410	de Chambrier et al. 2015
Amphoteromorphus piraeeba Woodland, 1934	*Brachyplatystoma filamentosum*	22227	KP729407	de Chambrier et al. 2015
Amphoteromorphus piriformis Carfora, de Chambrier et Vaucher, 2003	*Brachyplatystoma rousseauxii*	22211	AJ275231	de Chambrier et al. 2004a
Brayela karuatayi (Woodland, 1934)	*Platynematichthys notatus* (Jardine)	63128	KP729406	de Chambrier et al. 2015
Chambriella megacephala (Woodland, 1934)	*Sorubimichthys planiceps* (Spix et Agassiz)	91863–91865, 91867–91868, 69568, 72973	KY207449*	Alves et al. 2017b
Choanoscolex abscisus (Riggenbach, 1895)	*Pseudoplatystoma corruscans* (Agassiz)	17905	AJ388630	Zehnder and Mariaux 1999
Choanoscolex sp.	*Pseudoplatystoma fasciatum* (Linnaeus)	25102	AJ275064	de Chambrier et al. 2004a
Endorchis piraeeba Woodland, 1934	*Brachyplatystoma filamentosum*	21738	AJ388603	Zehnder and Mariaux 1999
Gibsoniela mandube (Woodland, 1935)	*Ageneiosus* sp.	63119	KP729412	de Chambrier et al. 2015
Gibsoniela meursaulti de Chambrier et Vaucher, 1999	*Ageneiosus inermis*	21839	AJ388631	Zehnder and Mariaux 1999
Goezeella siluri Fuhrmann, 1916	*Pinirampus pirinampu* (Spix et Agassiz)	21877	AJ388612	Zehnder and Mariaux 1999
Harriscolex kaparari (Woodland, 1935)	*Pseudoplatystoma tigrinum* (Valenciennes)	22018	AJ275227	Zehnder et al. 2000
Jauella glandicephalus Rego et Pavanelli, 1985	*Zungaru jahu* (Ihering)	31179	KP729399	de Chambrier et al. 2015
Megathylacus jandia Woodland, 1934	*Zungaro zungaro* (Humboldt)	21874	AJ388596	Zehnder and Mariaux 1999
Monticellia coryphicephala (Monticelli, 1891)	*Salminus brasiliensis* (Cuvier)	17984	AJ238832	Zehnder and Mariaux 1999
Monticellia ophisterni Scholz, de Chambrier et Salgado-Maldonado, 2001	*Ophisternon aenigmaticum* Rosen et Greenwood	-	AY307121	Scholz et al. 2003
Nomimoscolex admonticellia (Woodland, 1934)	*Pinirampus pirinampu*	21870	AJ388628	Zehnder and Mariaux 1999
Nomimoscolex chubbi (Pavanelli et Takemoto, 1995)	*Gymnotus carapo* Linnaeus	20351	AJ388625	Zehnder and Mariaux 1999
Nomimoscolex dorad (Woodland, 1935)	*Brachyplatystoma rousseauxii*	22269	AJ388613	Zehnder and Mariaux 1999
Nomimoscolex lenha (Woodland, 1933)	*Sorubimichthys planiceps*	21740	AJ388611	Zehnder and Mariaux 1999
Nomimoscolex lopesi Rego, 1989	*Pseudoplatystoma fasciatum*	21963	AJ388618	Zehnder and Mariaux 1999
Nomimoscolex matogrossensis Rego et Pavanelli, 1990	*Hoplias malabaricus* (Bloch)	17913	AJ388614	Zehnder and Mariaux 1999
Nomimoscolex piraeeba Woodland, 1934	*Brachyplatystoma capapretum* Lundberg et Akama	22284	AJ388608	Zehnder and Mariaux 1999
Nomimoscolex sudobim Woodland, 1935	*Pseudoplatystoma fasciatum*	21969	AJ388597	Zehnder and Mariaux 1999
Nomimoscolex suspectus Zehnder, de Chambrier, Vaucher et Mariaux, 2000	*Brachyplatystoma vaillanti* (Valenciennes)	22298	AJ388602	de Chambrier et al. 2004a
Nupelia portoriquensis Pavanelli et Rego, 1991	*Sorubim lima* (Bloch et Schneider)	34185	KP729401	de Chambrier et al. 2015
Ophiotaenia europaea Odening, 1963	*Natrix maura* (Linnaeus)	18407	AJ388598	Zehnder and Mariaux 1999
Ophiotaenia filaroides (La Rue, 1909)	*Ambystoma tigrinum* (Green)	63372	KP729416	de Chambrier et al. 2015
Ophiotaenia paraguayensis (Rudin, 1917)	*Hydrodynastes gigas* (Duméril, Bibron et Duméril)	16927	AJ388629	Zehnder and Mariaux 1999
Ophiotaenia cf. *perspicua* La Rue, 1911	*Nerodia rhombifer* (Hallowell)	35370	KP729415	de Chambrier et al. 2015
Ophiotaenia sanbernardinensis Rudin, 1917	*Helicops leopardinus* (Schlegel)	18251	AJ388637	Zehnder and Mariaux 1999
Ophiotaenia saphena Osler, 1931	*Lithobates pipiens* (Schreber)	32851	KP729402	de Chambrier et al. 2015
Peltidocotyle lenha (Woodland, 1933)	*Zungaro zungaro*	22373	AJ238837	Zehnder and Mariaux 1999
Peltidocotyle rugosa Diesing, 1850	*Pseudoplatystoma reticulatum* Eigenmann et Eigenmann	22374	AJ238835	Zehnder and Mariaux 1999
Proteocephalidae gen. sp.	*Amia calva* Linnaeus	35548	FM956088	de Chambrier et al. 2009
Proteocephalus perplexus La Rue, 1911	*Amia calva*	35366	FM956089	de Chambrier et al. 2009
Proteocephalus sp.	*Ictalurus punctatus* (Rafinesque)	36278	FM956085	de Chambrier et al. 2009
Regoella brevis Arredondo, Gil de Pertierra et de Chambrier, 2013	*Pseudoplatystoma reticulatum*	79184	KP729389	de Chambrier et al. 2015
Riggenbachiella amazonense Alves, de Chambrier, Luque et Scholz, 2017	*Sorubimichthys planiceps*	60046, 60048, 91866	KY207451**	Alves et al. 2017b
Spasskyellina lenha (Woodland, 1933)	*Sorubimichthys planiceps*	69600	KP729413	de Chambrier et al. 2015
Spasskyellina spinulifera (Woodland, 1935)	*Pseudoplatystoma corruscans*	34216	KP729417	de Chambrier et al. 2015
Spatulifer maringaensis Pavanelli et Rego, 1989	*Sorubim lima*	21986	AJ388634	de Chambrier et al. 2004a
Synbranchiella mabelae gen. n. et sp. n.	*Synbranchus marmoratus* Bloch	MACN-Pa 619/2	**KY798870**	Present study
Testudotaenia testudo (Magath, 1924)	*Apalone spinifera* (Le Sueur)	35320	FM956082	de Chambrier et al. 2009

† unless otherwise stated, all vouchers are deposited at the Natural History Museum, Geneva, Switzerland (acronym MHNG-PLAT); MACN-Pa – Parasitological Collection of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina; *seven identical replicates; **three identical replicates

Table 2. Microthrix pattern of *Synbranchiella mabelae* gen. n. et sp. n. from *Synbranchus marmoratus* Bloch.

Surfaces	Microthrix type	Size (length × width)	Fig. 4
ASS	CF	0.64–0.92 (0.79) × 0.10–0.16 (0.12) (n = 9)	E
MSS	AF-CF/GS	0.53–0.90 (0.73) × 0.08–0.15 (0.11) (n = 9) 0.92–1.13 (1.04) × 0.47–0.64 (0.56) (n = 8)	F
LSS	CF/GS	0.78–1.06 (0.92) × 0.12–0.16 (0.14) (n = 9) 1.00–1.37 (1.19) × 0.50–0.59 (0.57) (n = 5)	G
SSS	AF/gs/GS	0.62–0.83 (0.70) × 0.12–0.16 (0.14) (n = 6) 0.63–0.83 (0.70) × 0.33–0.42 (0.39) (n = 5) 1.08–1.16 (1.16) × 0.51–0.57 (0.55) (n = 5)	H
N-ASS (anterior-medial)	CF/GS	0.76–1.23 (0.95) × 0.10–0.17 (0.13) (n = 15) 0.81–1.67 (1.27) × 0.40–0.69 (0.58) (n = 9)	I
N-ASS (posterior)	AF/GS	0.43–0.69 (0.55) × 0.11–0.13 (0.12) (n = 5) 1.14–1.29 (1.22) × 0.60–0.66 (0.63) (n = 4)	J
PZS	AF	0.40–0.60 (0.47) × 0.11–0.14 (0.12) (n = 6)	K
IPS	AF	0.32–0.36 (0.33) × 0.10–0.11 (0.10) (n = 6)	L

Abbreviations: ASS – apical surface of the scolex; MSS – marginal surface of the suckers; LSS – luminal surface of the suckers; SSS – septum sucker surface; N-ASS – non-adherent surface of the suckers; PZS – proliferation zone surface; IPS – immature proglottid surface; AF – acicular filitriches; CF – capilliform filitriches; GS – gladiate spinitriches; gs – small gladiate spinitriches.

er Woodland, 1934. *Synbranchiella* gen. n. can be easily differentiated from *Choanoscolex*, *Manaosia*, *Monticellia*, *Regoella*, *Spasskyellina* and *Spatulifer* by its possession of biloculate, instead of uniloculate suckers. In *Ageneiella*, *Chambriella*, *Goezeella* and *Riggenbachiella*, the suckers are also biloculate, but the new genus can be distinguished from *Ageneiella* and *Goezeella* by the scolex morphology (quadrangular with a conical apex, without a metascolex *vs* massive and with a collar-like metascolex, respectively), the development of the internal longitudinal musculature (weakly developed *vs* strongly developed in the two latter genera), and the arrangement of the vitelline follicles (two narrow lateral bands *vs* two wide lateral bands [follicles only ventrally distributed in *Goezeella*], more concentrated posteriorly, with the ventral bands widened at ovary level) (see Fuhrmann 1916, de Chambrier and Vaucher 1999, de Chambrier et al. 2004b). In addition, the uterus of *Synbranchiella* is entirely cortical, whereas the uterine stem is cortical but some of the uterine diverticles penetrate the medulla in *Ageneiella* (see de Chambrier and Vaucher 1999).

Synbranchiella closely resembles *Chambriella* (syn. *Lenhataenia* de Chambrier et Scholz, 2008) and *Riggenbachiella* as recently characterised by Alves et al. (2017b). The new genus clearly differs from *Chambriella* and *Riggenbachiella* by the appearance of the scolex (subspherical to quadrangular in *Synbranchiella vs* quadrilobed, almost rectangular in apical view in the two latter genera), suckers (without free posterior margin overlapping the proliferation zone *vs* with free posterior margin in *Chambriella* and *Riggenbachiella*), in the presence of a conspicuous septum separating the two loculi of the suckers in *Synbranchiella*, combined with a robust and prominent non-adherent area of suckers (*vs* thinner and less conspicuous in *Chambriella* and *Riggenbachiella*), and the ovary in gravid proglottids (butterfly-shaped *vs* bilobed and slightly follicular in the latter two). Additionally, *Synbranchiella* possesses a cirrus-sac elongated to pyriform with a typical proteocephalidean sperm duct, whereas it is subovate with a thick-walled internal sperm duct in *Chambriella* and sigmoid, composed of three parts in *Riggenbachiella* (see de Chambrier and Scholz 2008, Alves et al. 2017b).

The study of the tegument surface of *Synbranchiella mabelae* gen. n. et sp. n. revealed the presence of three types of microtriches that are typical for proteocephalidean cestodes, acicular and capiliform filitriches and gladiate spinitriches distributed on the scolex, proliferation zone and proglottid surfaces (see Table 2). The microthrix pattern observed in *S. mabelae* seems to be slightly different from those in species of *Chambriella* and *Riggenbachiella*. Only two microthrix types (acicular and gladiate spinitriches) were observed on the apex of the scolex, the non-adherent surface of suckers and anterior margins of suckers in these genera (see fig. 2 in Alves et al. 2017b), even though the complete microthrix pattern is not known in species of *Chambriella* and *Riggenbachiella*.

Synbranchiella mabelae gen. n. et sp. n.

Figs. 1–4, Table 2

ZooBank number for species:
urn:lsid:zoobank.org:act:0AECC384-38EC-475F-B702-90F7D1F0F7CE

Description (based on two mature and one gravid specimens, transverse sections and two scolices studied using SEM from type locality). Proteocephalidae, Monticelliinae. Medium-sized worms, 28–88 mm (n = 3) in total length. Strobila acraspedote, flattened dorsoventrally, anapolytic, consisting of 37–67 (51; 3) immature proglottids (up to appearance of spermatozoa in vas deferens), 6–9 (8; 3) mature proglottids (up to appearance of eggs in uterus), 22 (1) gravid proglottids. Immature proglottids wider than long to longer than wide, 80–870 (465) × 400–690 (515; 17), length/width ratio 0.2–1.7 : 1. Mature proglottids longer than wide, 960–1,380 (1,150) × 400–870 (660; 12), length/width ratio 1.3–2.4 : 1. Gravid proglottids longer than wide, 1.58–2.52 mm (2.07 mm) × 500–960 (750; 5), length/width ratio 1.7–5.0 : 1 (Figs. 1, 2B,C).

Scolex quadrangular, formed by four lobes separated by grooves in apical view, 710–800 (755) × 710–840 (775; 2), wider than proliferation zone, bearing 4 biloculate suckers. Apex conical, without apical organ, with numerous gland-cells (Figs. 1, 2A, 4A,B,D). Suckers oriented anterolaterally, lacking free posterior margin, with loculi of unequal size, separating each other by a robust septum, anterior loculus 430–550 (490) × 230–320 (280), posterior loculus 300–340 (315; 7). Proliferation zone 460–560 (510) × 1.10–1.66 mm (1.33 mm; 2) (Figs. 1, 2A, 4A–D).

Apical surface of scolex (ASS) covered only with capiliform filitriches (Fig. 4E). Marginal surface of suckers (MSS) covered with acicular and capiliform filitriches interspersed with gladiate spinitriches (Fig. 4F). Luminal surface of suckers (LSS) covered with capilliform filitriches interspersed with gladiate spinitriches (Fig. 4G). Surface of septum of suckers covered with few acicular filitriches interspersed with gladiate spinitriches of two

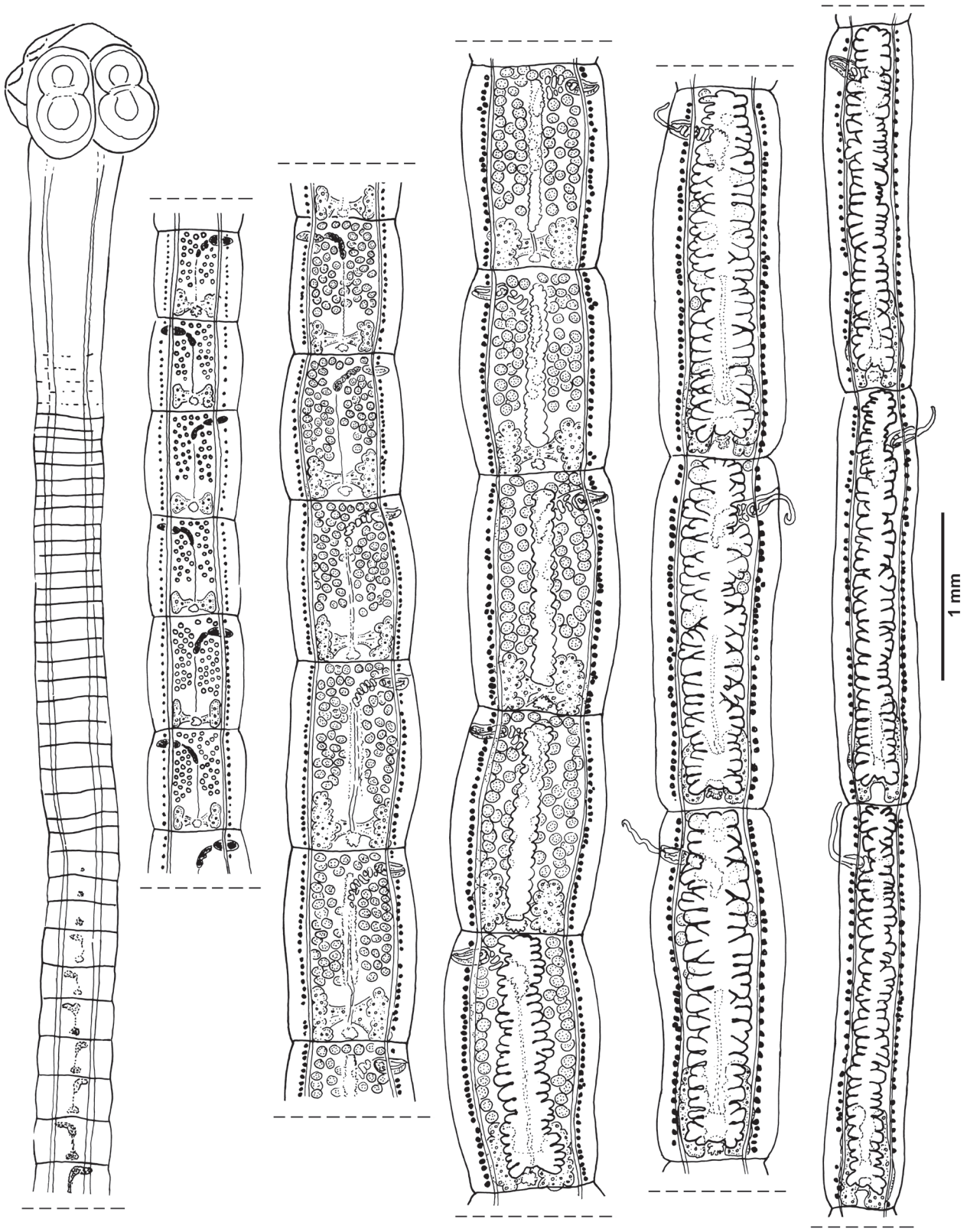

Fig. 1. *Synbranchiella mabelae* gen. n. et sp. n. from *Synbranchus marmoratus* Bloch (holotype IPCAS C-758). Entire worm, ventral view; dash lines indicate portions of strobila that are not shown.

sizes (Fig. 4H). Non-adherent surface of suckers (N-ASS) covered with capiliform filitriches interspersed with gladiate spinitriches on anterior and medial surfaces, filitriches diminishing in size from anterior to posterior surfaces (Fig. 4I,J). Proliferation zone surface and immature proglottid surface covered only with acicular filitriches (Fig. 4K,L). Tumuli observed in all surfaces, but more abundant on MSS, LSS and N-ASS (Fig. 4C,D) (see Table 2 for estimated size of microtriches).

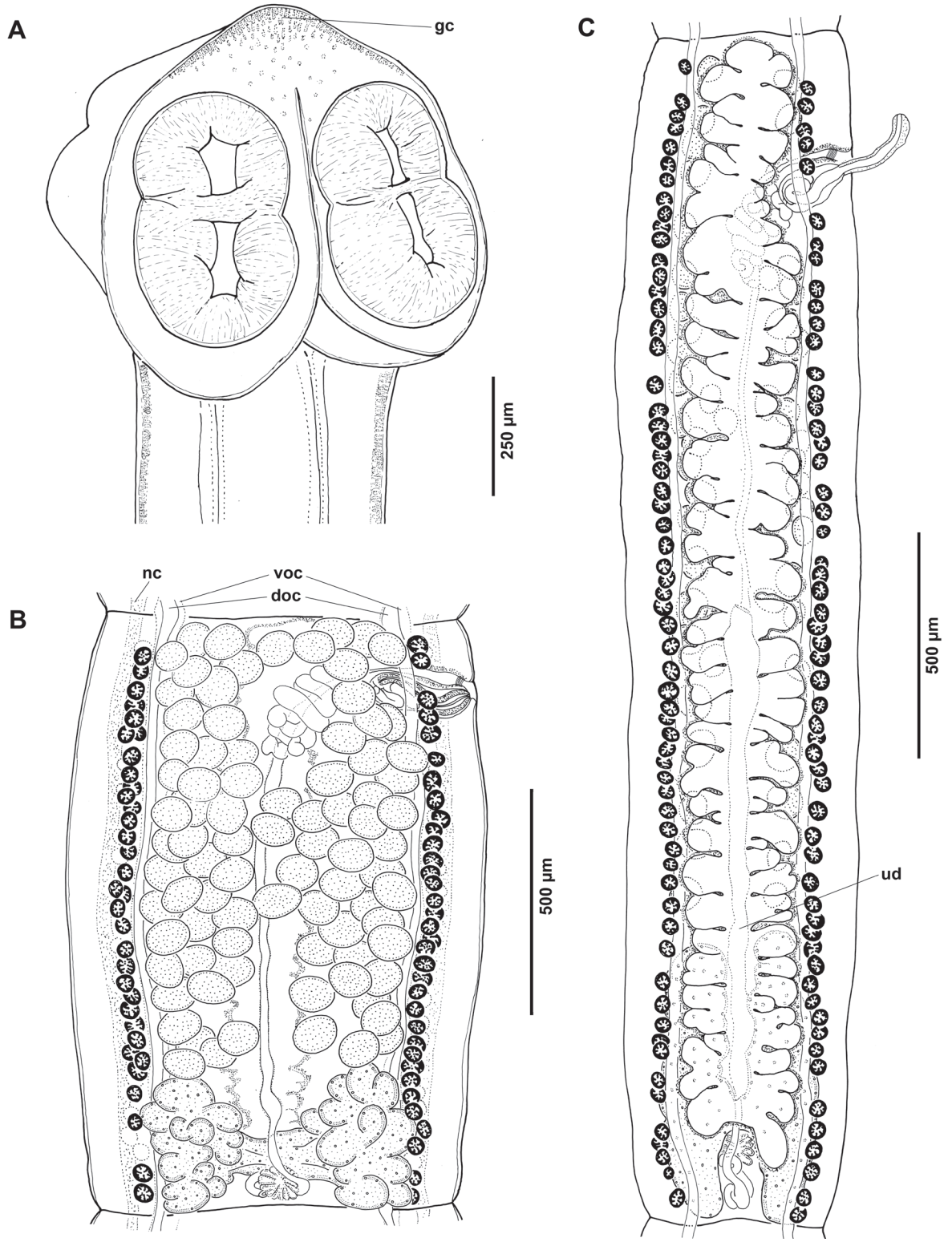

Fig. 2. *Synbranchiella mabelae* gen. n. et sp. n. from *Synbranchus marmoratus* Bloch (holotype, IPCAS C-758). **A** – scolex, dorsoventral view; **B** – mature proglottid, dorsal view; **C** – gravid proglottid, ventral view. *Abbreviations*: doc – dorsal osmoregulatory canal; gc – gland cells; nc – nerve corde; ud – uteroduct; voc – ventral osmoregulatory canal.

Internal longitudinal musculature weakly developed, represented by scarce bundles of isolated muscle fibres (Fig. 3D–F). Osmoregulatory canals situated between testes and vitelline follicles, often both canals overlapped by testes and ovary in dorsal view. Ventral canals 15–40 (30; 10) in diameter, dorsal canals 5–20 (15; 10) (Figs. 2B,C, 3D–F).

Testes cortical, oval to spherical 45–100 (75) × 40–80 (65; 25); 77–101 (88; 12) in total number per mature proglottid, arranged in one irregular field and one layer, usual-

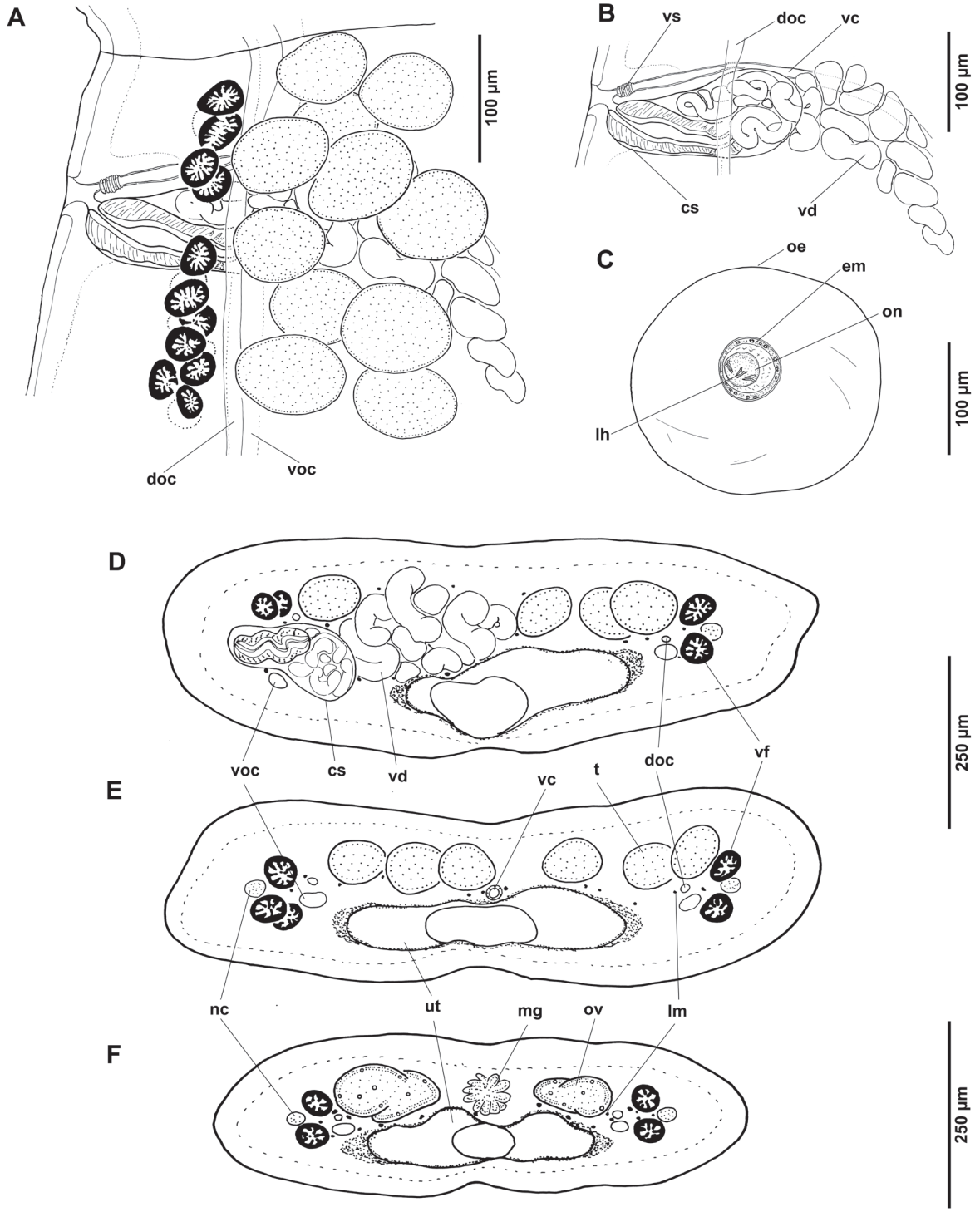

Fig. 3. *Synbranchiella mabelae* gen. n. et sp. n. from *Synbranchus marmoratus* Bloch (holotype, IPCAS C-758). **A** – detail of terminal genitalia, dorsal view; **B** – terminal genitalia (testes and vitelline follicles omitted), dorsal view; **C** – egg; **D** – cross-section at level of cirrus-sac; **E** – cross-section at level of testes; **F** – cross-section at level of ovary. *Abbreviations*: cs – cirrus-sac; doc – dorsal osmoregulatory canal; em – embryophore; lh – larval hooks; lm – longitudinal musculature; mg – Mehlis' gland; nc – nerve corde; oe – outer envelope; on – oncosphere; ov – ovary; t – testis; ut – uterus; vc – vaginal canal; vd – vas deferens; vf – vitelline follicles; voc – ventral osmoregulatory canal; vs – vaginal sphincter.

ly not surpassing osmoregulatory canals, overlapping cirrus-sac and ovary (Figs. 2B, 3A,D). Cirrus-sac elongate to pyriform, with thin muscular wall, 140–225 (200) × 65–90 (80; 12), occupying 24–42% (31%; n = 12) of proglottid width in mature proglottids. Cirrus long, occupies 52–79% (69%; 12) of cirrus-sac length in mature proglottids (Figs. 2B,C, 3A,B,D). Evaginated cirrus 325–415 (380) × 40–50 (45; 5). Vas deferens coiled, 15–40 (30; 21) in diameter, usually not surpassing mid-line in mature and gravid proglottids. Genital pores irregularly alternating, markedly pre-equatorial, 8–16% (12%; 12) from anterior margin of proglottid in mature proglottids (Figs. 1, 2B,C, 3A).

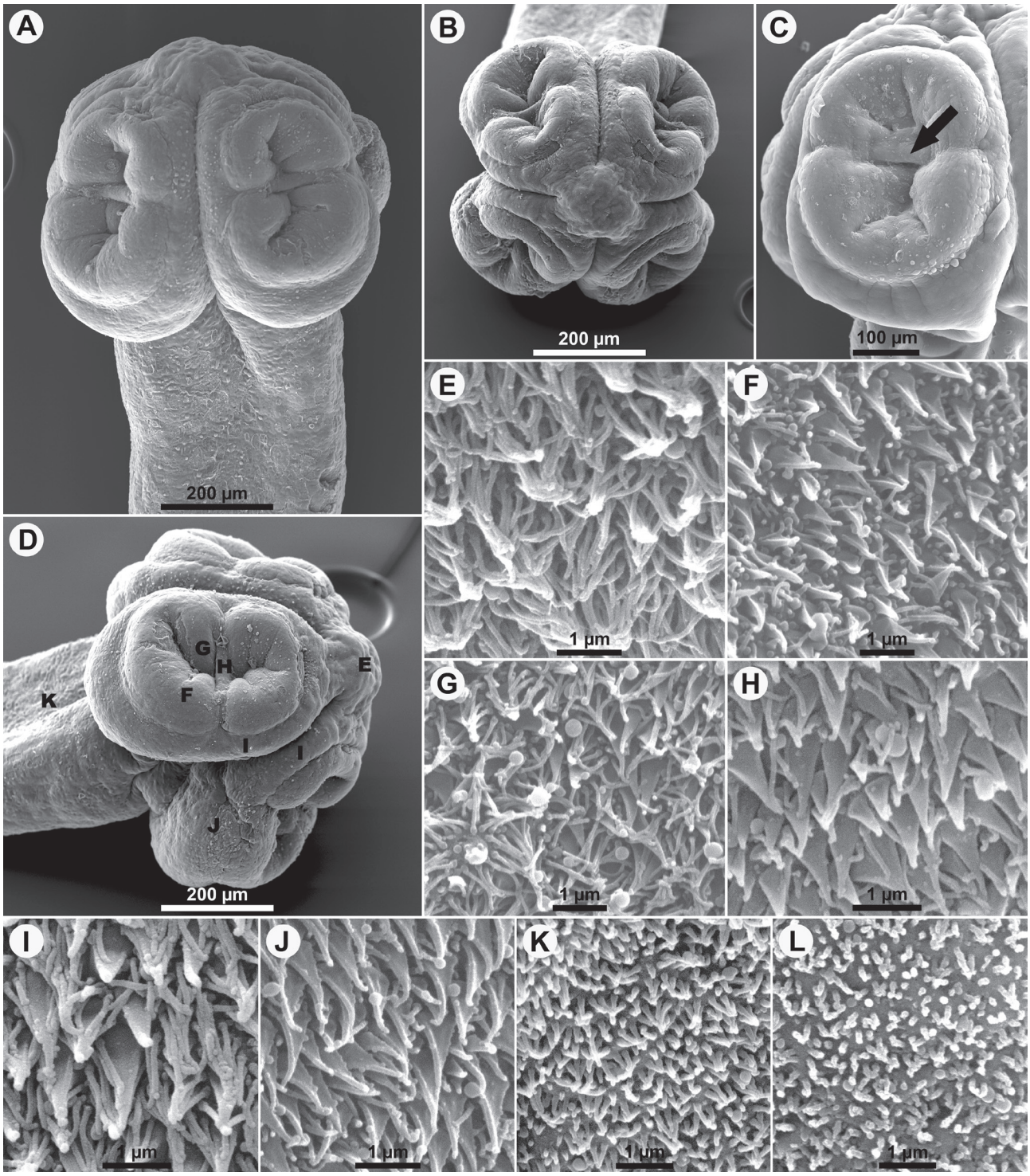

Fig. 4. *Synbranchiella mabelae* gen. n. et sp. n. from *Synbranchus marmoratus* Bloch, scanning electron micrographs. **A** – scolex, dorsoventral view; **B** – scolex, apical view; **C** – detail of sucker, arrow indicates septum; **D** – scolex, sublateral view; E–K letters indicate surfaces shown at high magnification in Fig. 4E–K; **E** – apical surface of scolex; **F** – marginal surface of suckers: **G** – luminal surface of suckers; **H** – surface of sucker septum; **I** – non-adherenth surface of suckers, anterior and medial zone; **J** – non-adherent surface of suckers, posterior zone; **K** – proliferation zone surface; **L** – surface of immature proglottid.

Ovary cortical, butterfly-shaped, slightly lobulate, 225–315 (280) × 235–590 (400; 12), occupying 47–72% (60%; 12) of mature proglottid width (Figs. 1, 2B,C, 3F). Relative size of ovary surface to proglottid surface (*sensu* de Chambrier et al. 2012) 8–14% (11%; n = 11). Vagina thin-walled, always anterior to cirrus-sac, with small vaginal sphincter (difficult to observe), 10–15 (13; 10) in diameter (Figs. 2B,C, 3A,B). Vitelline follicles cortical, arranged in 2 narrow lateral bands of 1–2 rows of follicles, occupy 97–100% of proglottid length. Some follicles overlapping ovary ventrally and vagina and cirrus-sac dorsally (Figs. 1, 2B,C, 3A, D–F).

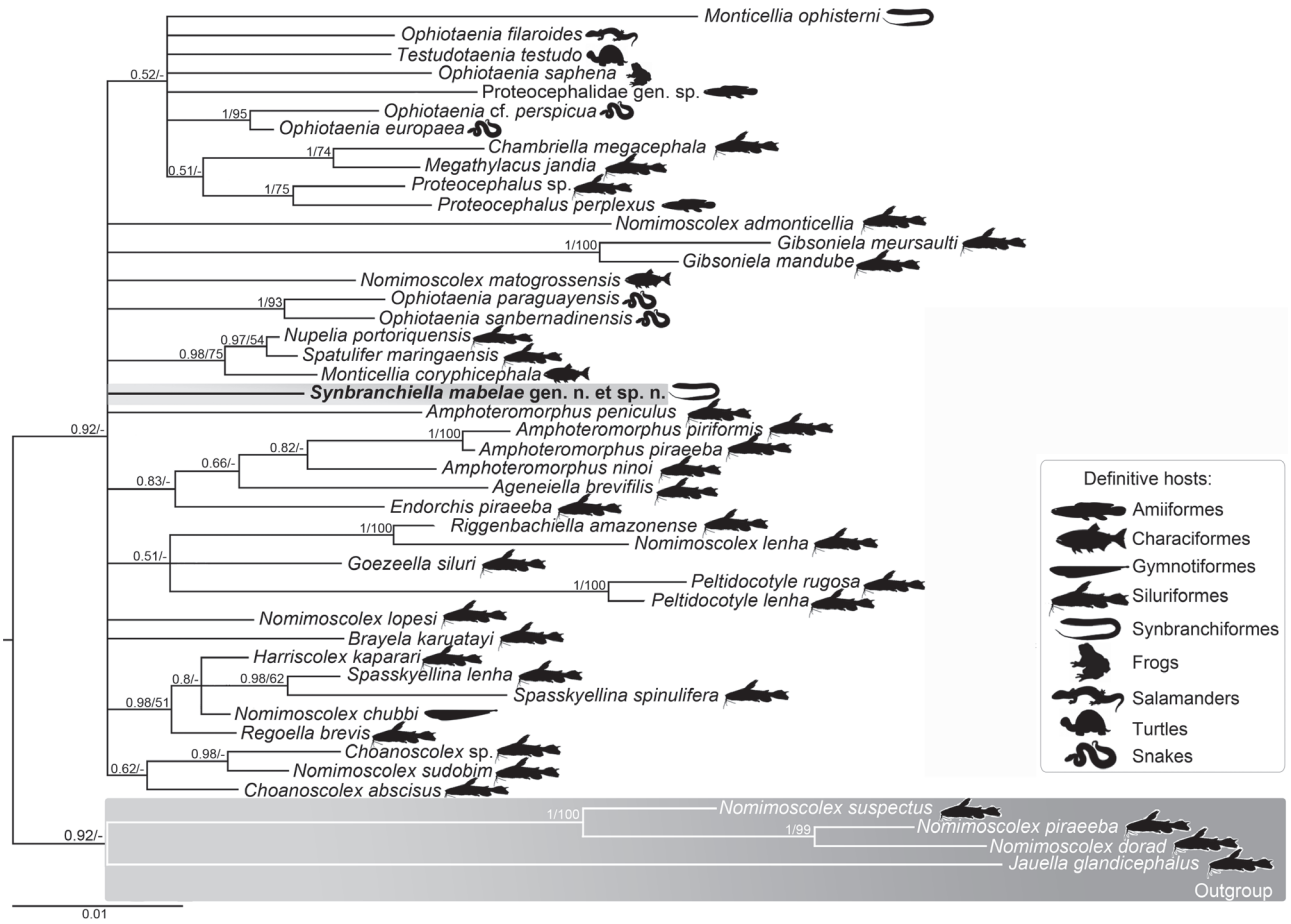

Fig. 5. Phylogram based on Bayesian Inference analysis of the partial *lsr*DNA data. Nodal values indicate Bayesian posterior probabilities > 0.5 and Maximum Likelihood bootstrap supports > 50. Branch length scale bar indicates number of substitutions per site. *Synbranchiella mabelae* gen. n. et sp. n. from *Synbranchus marmoratus* Bloch in bold and demarcated in grey.

Uterine stem cortical, uterine development of type 2 (*sensu* de Chambrier et al. 2004a). Uterus entirely cortical, uterine branches situated in ventral cortex, occupying 43–58% (53%; 5) of width of gravid proglottids. Aporal uterine branches 33–50 (40; 5) in number, poral uterine branches 34–48 (40; 5) in number. Uteroduct 760–1,000 (880) × 45–55 (50), occupying 35–41% (38%; 3) of gravid proglottid length (Figs. 1, 2C, 3D–F).

First eggs released through several circular uterine apertures, later through longitudinal slit-like aperture extending ventrally along almost entire length of proglottid. Eggs spherical, with thin hyaline outer envelope 115–175 (140; 16) in diameter; embryophore 36–45 (40; 16) in diameter; oncosphere 20–30 (24; 16) in diameter, with embryonic hooks 8–13 (10; 39) long (Fig. 3C).

Type and only known host: *Synbranchus marmoratus* Bloch (Synbranchiformes: Synbranchidae); vernacular name 'anguila criolla' in Argentina; marbled swamp eel in English.

Type locality: River Colastiné (tributary of the River Paraná; Middle Paraná), near Barrio Colastiné Sur, Santa Fe Province, Argentina (31°40'S; 60°46'W).

Other locality: River Paraná-Guazú (tributary of the River Paraná; Lower Paraná), Entre Rios Province, Argentina (33°54'S; 58°52'W).

Site of infection: Anterior intestine.

Infection rates: River Colastiné – prevalence, 17% (8/47), intensity 1–2 worms per host, mean intensity 1.3, abundance 0.2; River Paraná-Guazú – prevalence 19% (5/26), intensity 1–7 worms per host, mean intensity 2.4, abundance 0.5; total number of worms 22; 19 immature, 2 mature, 1 gravid.

Type material: Holotype IPCAS No. C-758/1 (entire worm with serial transverse sections, on three slides), paratypes: MACN-Pa 619/1A,B (entire worm with serial transverse sections on two slides), MACN-Pa 619/2 (hologenophore; scolex used for SEM micrographs, strobila with serial transverse sections on one slide).

Molecular data: A fragment of 1,497 bp of the *lsr*DNA gene (D1–D3 domains) of one specimen of *Synbranchiella mabelae* was amplified. The nucleotide sequence is available in the GenBank database (Accession No. KY798870).

Etymology: The species is dedicated to the first author's mother Mabel Vera who also helped with collection of fishes.

Phylogenetic analysis. Partial *lsr*DNA (D1–D3 domains) sequence was generated *de novo* for a single representative of *Synbranchiella mabelae* gen. n. et sp. n. The trimmed *lsr*DNA alignment that also included representatives of clade D of de Chambrier et al. (2015), *Monticellia ophisterni* Scholz, de Chambrier et Salgado-Maldonado, 2001 (the only Neotropical proteocephalidean described from a synbranchid host), as well as representative se-

quences of most morphologically similar species, i.e. *C. megacephala* and *R. amazonense* (see Table 1), was 976 bp long and included 136 parsimony informative characters.

Bayesian inference and Maximum likelihood analyses produced phylograms with similar topologies (Fig. 5), even though weaker supported in the ML data (data not shown). The results showed a large polytomy with few well-supported internal nodes. Nevertheless, the molecular results revealed *S. mabelae* as an independent lineage, yet with uncertain phylogenetic position among the Neotropical proteocephalideans (Fig. 5). The morphologically similar taxa, *C. megacephala* and *R. amazonense*, clustered together with *Megathylacus jandia* Woodland, 1934 and *Nomimoscolex lenha* (Woodland, 1933), respectively, whereas *M. ophisterni* fell within a weakly supported clade composed, among others, from several species of *Ophiotaenia* La Rue, 1911 from amphibians and reptiles in the Palaearctic and Nearctic regions, also with unresolved position.

Pairwise comparison of the *lsr*DNA sequences of *S. mabelae* with those of *R. amazonense*, *C. megacephala* and *M. ophisterni* revealed divergence levels of 2.6% (39 nt difference), 2.7% (40 nt difference) and 4.8% (48 nt difference), respectively.

DISCUSSION

Synbranchiella mabelae gen. n. et sp. n. belongs to the Monticeliinae based on the cortical position of the testes, ovary, vitelline follicles and uterus, as defined by Schmidt (1986), Rego (1994) and de Chambrier et al. (2009). The new species is allocated in a new genus because it possesses a unique combination of characters not present in any other monticelliine genera.

Recently, Caira et al. (2014) proposed the presence of gladiate spinitriches on the proliferation zone (or neck) of the Proteocephalidea and in the cephalic peduncle of the Onchobothriidae as a synapomorphy of the Onchoproteocephalidea. However, the type of microtriches that covers the proliferation zone has been scarcely included in the descriptions of proteocephalidean species and thus future studies should test validity of this putative synapomoprhy of the Onchoproteocephalidea. For example, the new species described herein, *Spatulifer maringaensis* Pavanelli et Rego, 1989 and *Luciaella ivanovae* Gil de Pertierra, 2009 have not gladiate spinitriches covering the proliferation zone (Arredondo and Gil de Pertierra 2008, Gil de Pertierra 2009, present study).

Phylogenetic analysis of the partial *lsr*DNA sequence of *Synbranchiella mabelae* shows that this species does not cluster with any other Neotropical proteocephalidean, even though its relationship with the taxa remains unclear. It also indicates that the most morphologically similar taxa, i.e. *C. megacephala* and *R. amazonense*, are reciprocally monophyletic lineages, also with uncertain position within a large polytomy (see Fig. 5). It is argued that several events of colonisation of both hosts and zoogeographical regions, associated with rapid radiation in Neotropical teleosts, mainly pimelodid catfishes, largely contributed for the lack of genetic signal as estimated on the basis of the current ribosomal data (de Chambrier et al. 2004a, 2015). The results obtained in the present study support this assumption this assumption, as revealed by the low divergence levels of the *lsr*DNA sequences, at least among representatives of three genera, i.e. *Chambriella*, *Riggenbachiella* and *Synbranchiella*; divergence levels ranged between 2.6%–4.8% (39–48 nt difference).

All three monticelliine genera possessing biloculate suckers are morphologically similar, but it is obvious from molecular analyses that this resemblance is a result of convergent evolution of morphological traits. Homoplasy of morphological characteristics, especially those of the scolex, has been observed in several groups of proteocephalideans [e.g. Scholz et al. 2013 – *Macrobothriotaenia ficta* (Meggitt, 1931)]. In South America, there are two other proteocephalidean genera from fishes that do not possess a metascolex and that bear four biloculate suckers in the scolex, similar to the members of the three above-mentioned monticelliine genera, i.e. *Endorchis* Woodland, 1934 (Endorchiinae) and *Luciaella* Gil de Pertierra, 2009 (Peltidocotylinae). Preliminary analyses of molecular data (partial sequences of *lsr*DNA) support the assumption that biloculate suckers may have evolved independently in several lineages of proteocephalidean cestodes in the Neotropical Region (P.V.A. – unpubl. data).

Neotropical synbranchids are represented by two species of *Ophisternon* McClelland, namely *O. aenigmaticum* Rosen et Greenwood and *O. infernale* (Hubbs) distributed in Central America, and three species of *Synbranchus* Bloch, i.e. *S. marmoratus*, *S. lampreia* Favorito, Zanata et Assumpção and *S. madeirae* Rosen et Rumney, distributed in Central and South America (Froese and Pauly 2016). Only one proteocephalidean, *Monticellia ophisterni*, was previously found in synbranchids in the Neotropical Region (Scholz et al. 2001). *Synbranchiella mabelae* occurs in *S. marmoratus*, which has the most widespread distribution among the Neotropical synbranchids (Central and South America). *Monticellia ophisterni* differs from *S. mabelae* especially in the possession of uniloculate rather than biloculate suckers (see Scholz et al. 2001). Dissimilarity of these two monticelliine cestodes from swamp eels (synbranchiform fishes) well corresponds to their low level of relatedness as revealed by the phylogenetic analyses (Fig. 5) and distant distribution areas (northern Argentina *vs* southeastern Mexico). It is thus plausible to assume that synbranchiform fishes in the Neotropical Region were colonised by proteocephalidean cestodes independently. Curiously, *M. ophisterni* and *S. mabelae* have a relatively high prevalence of infection but most specimens were not fully mature or gravid (see Scholz et al. 2001 and infection rates in this paper). According to Scholz et al. (2001), the occurrence of *M. ophisterni* in an eel could be a result of host-switching, since other species of *Monticellia* have mainly been reported from siluriform or characiform fishes. Thus, the presence of proteocephalidean species in synbranchid fishes could either reflect a recent acquisition or an accidental host. *Synbranchiella mabelae* is the second proteocephalidean cestode described from a Neotropical

synbranchiform fish host and the first one in South America.

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